

Claims.

1. A method of determining the degree of similarity between gene expression in a biological sample of interest and that in individual reference samples, comprising
 - (a) providing a nucleic acid probe library representative of a pattern of gene expression in the biological sample of interest,
 - (b) providing a plurality of reference samples each being a nucleic acid library representative of a pattern of gene expression in reference biological samples from which the reference samples have been derived,
 - (c) forming a first set of immobilised, hybridised products by treating the individual reference samples with the probe library under hybridising conditions, one or other of the reference samples or the probe library being in immobilised form, and removing non-immobilised material,
 - (d) forming a second immobilised product by treating a sample of the free probe library with an immobilised sample of the probe library under hybridising conditions, and removing non-immobilised material,
 - (e) effecting progressive dissociation of the hybridised products obtained in steps (c) and (d),
 - (f) monitoring said progressive dissociation, and
 - (g) comparing the results of step (f) for the hybridised products obtained in step (c) with those obtained for the hybridised products obtained in step (d) to determine said degree of similarity.
2. A method according to claim 1, wherein dissociation of the hybridised samples is brought about by exposing the samples to increasing temperature.
3. A method according to claim 1 or claim 2, wherein dissociation of the hybridised samples is brought about by exposing the samples to increasing concentrations of chemical denaturants.

4. A method according to any preceding claim wherein dissociation is monitored using a marker capable of differentiating between double-stranded and single-stranded nucleic acids.
5. A method according to claim 4, wherein dissociation is monitored using ethidium bromide.
6. A method according to claim 4, wherein dissociation is monitored using SybrGreen.
7. A method according to any preceding claim, wherein dissociation is monitored by detecting the generation of single stranded nucleic acids on dissociation of double stranded hybridised material.
8. A method according to any preceding claim, wherein dissociation is monitored using probe library and reference samples labelled with markers capable of generating a signal when the markers are in proximity to one another that can be distinguished from that signal generated when the markers are distant from one another.
9. A method according to any preceding claim, wherein dissociation is monitored using a labelled non-immobilised nucleic acid population and an unlabelled immobilised nucleic acid population.
10. A method according to claim 9, wherein dissociation is monitored by assessing the residual label retained by the immobilised material on removal of non-immobilised material.
11. A method according to claim 9, wherein dissociation is monitored by assessing the labelled material released from the immobilised material.
12. A method according to any of claims 4 to 11, wherein dissociation is monitored using a fluorescent marker.

13. A method according to any preceding claim, wherein the reference samples are provided as an array on a substrate.
14. A method according to any preceding claim, wherein the reference samples comprise cDNA or a derivative thereof derived from biological reference samples representing a number of different biological conditions or states.
15. A method according to any preceding claim, wherein the reference samples comprise cDNA or a derivative thereof derived from biological reference samples representing a number of different examples of the same biological condition or state.
16. A method according to any preceding claim, wherein the probe library is prepared by a complexity reduction technique from cDNA obtained from the biological sample of interest.
17. A method according to any preceding claim, wherein the reference samples are prepared by a complexity reduction technique from cDNA obtained from the reference biological samples.
18. A method as claimed in claim 16 or claim 17, wherein the complexity reduction technique comprises a restriction digestion technique.
19. A method as claimed in claim 16 or claim 17, wherein the complexity reduction technique comprises a subtraction technique.
20. A method as claimed in claim 16 or claim 17, wherein the complexity reduction technique comprises a cDNA display technique.
21. A method as claimed in any preceding claim, wherein the hybridisation is effected in the presence of competitor DNA.

22. A method according to any preceding claim, wherein the probe library is labelled with a fluorophore in order to determine the relative degree of hybridisation of the probe library to the reference samples.
23. A method according to any preceding claim, wherein the probe library or reference samples are subject to partial exonuclease digestion prior to effecting hybridisation.
24. A method according to claim 23, wherein both the probe library and the reference samples are subject to partial exonuclease digestion prior to effecting hybridisation, and the probe library and reference samples are treated with exonucleases having different specificities.